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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

In the concluding year of support, we have focussed on the development of Computer Simulations on the counterion atmosphere of DNA using Monte Carlo methods. Canonical ensemble simulations were carried out to obtain a description of the counterion distribution around DNA as a function of variations on the primitive model for interparticle interactions. Grand Canonical Monte Carlo simulations were carried out to study thermodynamic properties for simple and polyelectrolyte solutions. An account of the contravariant behavior of activity coefficient as a function of concentration in simple vs. polyelectrolyte solutions has been obtained. The structure of the ion atmosphere has been examined and provides insight into structuration effects at the onset of the breakdown in Debye Huckel theory.

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Date: April 1, 1990

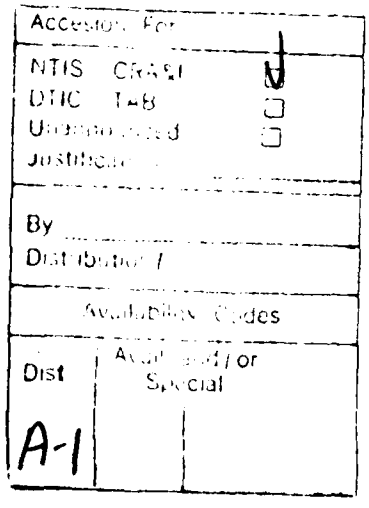
Final Report on Contract N00014-87-K-0312

Principal Investigator: Prof. D. L. Beveridge

Contractor: Wesleyan University

Title: ENVIRONMENTAL EFFECTS AND OLIGONUCLEOTIDE STRUCTURE

Starting Date: June 1, 1987



I. Project Goals and Overview

The object of this series of studies was to develop a theoretical account of the observed thermodynamic properties of simple and polyelectrolyte solutions based on molecular simulation, and to gain a structural perspective on the sources of nonideality in these mixtures, leading to the development of workable theoretical models to understand DNA-ligand interactions in aqueous solutions of simple electrolytes. The explicit treatment of DNA, water and counterions together in simulation has just now become feasible on present generation supercomputers, but such calculations at concentrations of simple salt and DNA of biophysical interest and experimental accessibility are still out of reach. This has led us and others to take up calculations in what is called the "primitive model," considering DNA and ions of the simple electrolyte explicitly with the solvent water given some kind of continuum treatment. One advantage of this approach is that simulations in the grand canonical Monte Carlo formalism are feasible and direct determination of excess chemical potentials and other thermodynamic properties can be carried out. The projects are still quite computationally intensive, however, and requred access to supercomputers. A program GCMC optimized for the CRAY-YMP has been prepared and implemented at the Pittsburgh Supercomputer Center.

In the concluding year of the project, studies have dealt with describing the counterion atmosphere of DNA using variations on the primitive model and optimization procedures to produce good thermodynamic properties of 1:1 simple electrolyte solutions. The efforts have been successful and have been extended to a treatment of DNA in excess 1:1electrolyte. A large MC/MD simulation on a 12 base pair DNA sequence including 2000 water molecules and 22 counterions has been completed.

II. Results

Ion atmosphere of DNA. Canonical Monte Carlo simulations on aqueous solutions of sodium salt of DNA at different added sodium chloride concentrations have been carried out, at a temperature of 25°C in a (T,V,N) ensemble (Macromolecules, in press). The detailed shape of the DNA, the finite size and spatial

correlations of the counter- and co-ions are taken into account. Convergence criteria, step size, single versus multi-particle moves and other methodological issues in the Monte Carlo calculations are addressed. The potential influence of the dielectric discontinuity between the DNA and the solvent and the implications of dielectric saturation around DNA were examined. We find in all cases a concentration of counterions near DNA (~10Å) that is in excess of 1M even in the absence of excess salt, consistent with the counterion condensation model. A consideration of the lowering of the dielectric constant near DNA due to dielectric saturation in water results in an essentially salt independent estimate of the net counterionic charge per phosphate around DNA, over the added salt concentration range studied. This observation is also consistent with the counterion condensation theory and the current inferences from ^{23}Na NMR experiments.

Simple electrolyte solutions. We subsequently carried out grand canonical Monte Carlo simulations on aqueous solutions of sodium chloride, at a temperature of 25°C in a (T,V,μ) ensemble, using a refined primitive model (J. Phys. Chem., submitted). The ions interact with each other in a solvent treated as a dielectric continuum, via [12,6,1] potentials. Our big breakthrough came when a Gurney term was added to this energy function to consider desolvation of two encroaching ions. This refined primitive model has satisfactorily accounted for the experimentally observed activity coefficient data of aqueous solutions of sodium chloride at 25°C over a concentration range of 5 mM to 500 mM, far beyond that of most of the previous theoretical treatments. Analysis of our results led to an enhanced perspective on the successes (below 10 mM) and failures (above 10 mM) of the Debye-Huckel theory of interionic interactions for simple electrolyte solutions. An interesting finding emerging from these calculations is that the above refined primitive model predicts the stability of the anionic pairs at high salt concentrations (>50 mM) found previously from detailed simulations with explicit solvent molecules and thought to be an impossibility in calculations with a continuum solvent.

Polyelectrolyte solutions. Encouraged by our success in predicting both the structural and thermodynamic properties of $[NaCl]_{aq}$, and with the experience gained from our canonical Monte Carlo studies on NaDNA in the presence of added sodium

chloride, we have extended our Grand Canonical Monte Carlo studies to [NaDNA + $NaCl]_{aq}$. The calculated and experimentally observed counterion activity coefficients differed only by 2-5%, at a DNA phosphate concentration of 2.89 mM and in the added salt concentration range of 0.85-2.65 mM. These studies were then extended to higher DNA concentrations (up to 31.1 monomolar) as well as higher NaCl concentrations (up to 200 mM) to determine excess chemical potentials, mean ionic activity coefficients of the added salt, preferential interaction coefficients (Donnan salt factor). Analysis of our results has enabled us to further an understanding of the sources of non-ideality in these ternary mixtures of sodium salts of DNA in the presence of sodium chloride.

III. Molecular Dynamics of a DNA Dodecamer Including Counterions and Water. In a parellel initiative, we have been carrying out molecular dynamics simulations on oligonucleotide sequences of DNA with a fully explicit consideration of counterions and water. We have found it necessary to conduct an extensive Monte Carlo pre-equilibration of the solvent to obtain stable MD simulations of the hydrated complex. At this point we have completed a 140 psec MD trajectory on the Eco-RI sequence d(CGCGAATTCGCG) which we find to remain in a stable B form throughout the simulation. A detailed comparison of the calculated results with a corresponding crystal structure and the results of several NMR investigations is currently being carried out. The sequence dependent behavior of the dynamical fine structure is being characterized in light of seveal proposed models for DNA bending.

IV. Significance

The structure and interactions of DNA are well known to be sensitive to environmental salt concenttraion; added salt in the case of GC rich sequences can even change the sense of the double helix from right- to left-handed! It has been hypothesized that regulatory events in molecular genetics may be highly sensitive to local ionic concentrations in the cell. Our theoretical understanding of salt effects and the ion atmosphere of DNA is now susceptible to study via molecular simulation, and it is important to the topics of drug-DNA and protein-DNA interactions to have an accurate description of these phenomena to further our basic understanding of the

structure and function of DNA in biological systems.

V. Publications (concluding year):

1. "Monte Carlo Simulation Studies of the Structure of the Counterion Atmosphere of D-NDA: Variations on the Primitive Dielectric Model," B. Jayaram, B. Honig, K. Sharp, S. Swaminathan and D. L. Beveridge, Macromolecules, in press.

2. "Grand Canonical Monte Carlo Simulations on Aqueous Solutions of NaCl and NaDNA: Excess Chemical Potentials land Sources of Non-ideality in Electrolyte and Polyelectrolyte Solutions," B. Jayaram and D. L. Beveridge, J. Phys. Chem., submitted.

3. "Molecular Dynamics of a DNA Dodecamer Including Counterions and Water," S. Swaminathan and D. L. Beveridge, J. Am. Chem. Soc., MS in preparation.

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12 PERSONAL AUTHOR(S): Beveridge, David L.

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The objective of this project is to carry out a critical and comparative study of the various theoretical models used for the treatment of environmental effects -- hydration and counterion atmosphere -- in molecular simulations on oligonucleotide systems. These studies form the basis for a series of collaborations with NMR spectroscopitsts and crystallographers on specific application areas of current interest in the field of nucleic acids research.

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PRINCIPAL INVESTIGATOR: Prof. D. L. Beveridge

CONTRACTOR: Wesleyan University

CONTRACT TITLE: Environmental Effects on Oligonucleotide Structure

STARTING DATE: June 1, 1987

Brief Summary of Project Goals

This project involves application of molecular simulation: molecular dynamics and Monte Carlo calculations to the structure, dynamics and solvation of oligonucleotide systems. Environmental effects are included in the simulations in a series of models involving progressively greater dimensionality, beginning with linear and sigmoidal forms of distance dependent dielectric functions representing the effect of water, and currently conventional screened charge models for the effect of counterions. The next level of rigor involves explicit consideration of oligonucleotide plus water with implicit (screened charge) counterion atmosphere and vice versa, explicit counterions with implicit (polarizable dielectric) water. Ultimately supercomputer simulations involving explicit representations of oligonucleotide, water and counterions will be studied, with special attention to the important but unexplored (and seemingly underappreciated) convergence issues in the calculations at this level. We hope to develop and characterize a protocol for optimum simulation methodology

required to produce reliable, reproducible results. Detailed comparison of the nucleic acid dynamics for these different environmental models will be developed also in terms of conformational and helicoidal analysis. Hydration and counterion atmosphere will be analyzed based on stereographic display of solvation density and the systematic partitioning of the complex results into well-defined contributions from the major groove, minor groove and sugar-phosphate backbone entities of the duplex. Thus a complete characterization of dynamical structure and solvation will be achieved for each system studied. These studies form the basis for a series of collaborations with NMR spectroscopists and crystallographers on specific application areas of current interest in the field of nucleic acid research.

Summary of Accomplishments: 6/1/87 - 5/31/88

In the first year of this project, two papers have been published and two more are submitted. Several additional papers are currently in preparation. In addition, two review articles on related subject areas have been prepared. In the following pages, the abstracts of the publications are collected, followed by brief descriptions of the projects currently in progress.

"Theoretical Considerations of the 'Spine of Hydration' in the Minor Groove of d(CGCGAATTCGCG)$_2$: Monte Carlo Computer Simulation," P. S. Subramanian, G. Ravishanker and D. L. Beveridge, *Proc. Natl. Acad. Sci.* **85**, 1836 (1988).

A theoretical description of aqueous hydration in the minor groove of a B-form DNA is presented here on the basis of a liquid state Monte Carlo computer simulation on a system consisting of the oligonucleotide duplex d(CGCGAATTCGCG)$_2$ in a canonical B-form together with 1777 water molecules contained in a hexagonal prism cell and treated under periodic boundary conditions. The results are analyzed in terms of solvent density distributions. The calculated minor groove solvent density shows considerable localization, indicative of discrete solvation sites and providing theoretical evidence for a well-defined ordered water structure. In the AATT sequence,

this corresponds to the 'spine of hydration' discovered by R. E. Dickerson and coworkers in the x-ray crystal structure of the dodecamer. We find however that the calculated ordered water structure also extends into the CGCG flanking sequences, supported by the N2 hydrogen bond donors and indicating that the 'spine of hydration' could thus extend throughout the minor groove of a B-form DNA. This provides a possible explanation of the positive binding entropies observed by K. J. Breslauer and coworkers for both AT and GC sequences on the complexation of netropsin to the minor groove of DNA's. Implications of these results with regard to the thermodynamic stability of DNA in water and the sequence specificity of the minor groove hydration are discussed.

"A Systematic Study of Patterns of Hydration in Nucleic Acids I. Guanine and Cytosine," H. M. Berman, A. Sowri, S. Ginell and D. L. Beveridge, *J. Biomol. Struct. Dyn.* **5**, 1101 (1988).

The hydration sites of guanine and cytosine are defined by examination of the crystal structures of bases, nucleosides, nucleotides, and three dinucleoside phosphate salts. Superposition of the results clearly reveals clusters of water molecules around certain of the heteroatoms of the bases. For the major groove region guanine, the G-N7 acceptor site shows two distinct populations of ordered water molecules, one above and one below the base plane. The G-06 shows only one population, more or less in plane. In the minor groove region, hydration sites appear at the G-N3 acceptor site and the G-N2 hydrogen bond donor, with these positions simultaneously occupied in the crystal. For cytosine in the GpC salts, hydration was observed only at the C-O2 acceptor site in the minor groove. The observed ordered water positions for both C and G in CpG, where observed, are well localized into sites and imply markedly limited ranges for solute-solvent hydrogen bond angles. Analysis of crystals containing only single C's and G's shows a broader range of hydrogen bond angles, and no particular clustering. The patterns of hydration for two guanine and cytosine containing oligonucleotides are then predicted. The relationship between these structural motifs and thermodynamic parameters is discussed.

"Free Energy *via* Molecular Simulation: A Primer," D. L. Beveridge and F. M. DiCapua, in *Computer Simulations in Protein Engineering and Drug Design,* P. Weiner, ed., in press (1988).

The various methods for free energy determination -- e.g., thermodynamic integration, the perturbation method, and the potential-of-mean-force -- are described in an elementary treatment of the subject in the context of molecular dynamics and Monte Carlo calculations on chemical and biomolecular systems. The theory, methodology and a representative application are described in each case. The capabilities and limitations of each of the methods are delineated.

"Free Energy *via* Molecular Simulation: Applications to Biomolecular Systems," D. L. Beveridge, *Ann. Rev. Biophys. Chem.* in press (1988).

The field of free energy simulations *circa* 1988 is reviewed as applied to biomolecular systems with an emphasis on the perturbation method and the thermodynamic cycle approach to substrate and drug binding to macromolecules. Recent studies are discussed and the capabilities and limitations of the method are critically considered, with particular regard to quasi ergodic problems. Future prospects for free energy simulations vis-a-vis the supercomputer era are explored.

"Aqueous Hydration of $r(GpC)_2$ at 25°C: Monte Carlo Computer Simulation," S. Pitchumani, P. S. Subramanian, D. L. Beveridge and H. M. Berman, *Biopolymers*, submitted.

Monte Carlo calculations are described for the dinucleotide duplex $r(CpG)_2$ together with 562 TIP4P water molecules at 25°C under periodic boundary conditions. The results are analyzed based on the proximity method and computer graphic presentations of hydration density distributions. Localization of hydration density is found to correspond closely to ordered water positions in C and G containing crystal hydrates. Discrepancies of commission at C-N4 and omission at G-N3 are noted and discussed in detail.

"Convergence Characteristics of Monte Carlo Free Energy Simulations using the Perturbation Method," P. S. Subramanian and D. L. Beveridge, *Theor. Chem.*, submitted.

The convergence characteristics of Monte Carlo simulations using the perturbation method are discussed in the context of a triangular thermocycle for the $\Delta\Delta G$ of hydration for ethane, methanol and methylamine. Error bounds are critically established using the method of batch means. Independent confirmation of the $\Delta\Delta G$ for the mutation of ethane to methanol was achieved and closure of the thermocycle within 0.4 kcal/mol was obtained.

Projects in Progress, 1988-89

•Monte Carlo Calculations of Hydration Density Distributions in A, B, and Z DNA Oligonucleotides: Comparison with Crystallographically Ordered Water Sites;

•Theory and Mechanism of the Hydration-Dependent A to B Conformational Transition in DNA;

•Molecular Dynamics of the dCpG/proflavin Crystal Hydrate (collaboration with Dr. H. M. Berman, Fox Chase Cancer Center);

•Monte Carlo Simulation of Ccunterion Condensation on DNA: Dielectric Function, Cation Type and Salt Dependence;

•An Atomic Solvation Theory for Nucleic Acids;

•Graphical Analysis of Microscopic Details of DNA Dynamics in Conformational and Helicoidal Coordinate Space (collaboration with Dr. R. Lavery, Institut de Biologie Physico-Chimique, Paris);

•Characterization of the Molecular Dynamics of Oligonucleotide Double Helices as Described by AMBER, CHARMm and GROMOS Force Fields; Influence of Dielectric, Counterion and Hydration Models;

•Determination of Oligonucleotide Structure in Solution Using 2D-NOE Distance Information from NMR and Restrained Molecular Dynamics Calculations (collaboration with Prof. P. H. Bolton);

•Effects of Hydration, Salt and Flanking Sequence Variations on the Structure and Dynamics of the Eco R1 Binding Site in the d(CGCGAATTCGCG) Duplex: Comparison of Results with NMR Studies (collaboration with Prof. I. Russu);

•Theoretical Studies of Molecular Recognition and Subunit Assembly Processes in ATCase (collaboration with Prof. N. Allewell).